Autosomal Dominant Central Areolar Choroidal Dystrophy Caused by a Mutation in Codon 142 in the Peripherin/RDS Gene

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• PURPOSE: Because several macular dystrophies are caused by mutations in the peripherin/RDS gene, we examined autosomal dominant and sporadic cases of central areolar choroidal dystrophy for mutations in the peripherin/RDS gene.

• METHODS: DNA sequence analysis of the peripherin/RDS gene was performed in four sporadic cases and in ten affected and nine unaffected individuals from seven families with autosomal dominant central areolar choroidal dystrophy.

• RESULTS: An Arg-142-Trp mutation in the peripherin/RDS gene was found in ten affected family members in seven families. Among these, a 69-year-old man with the Arg-142-Trp mutation, who was unaffected six years before blood sample analysis, showed a parafoveal area of chorioretinal atrophy. The 65-year-old sister of this family had the Arg-142-Trp mutation with no macular abnormalities, but she might still develop central areolar choroidal dystrophy at an older age. No mutation was found in the four sporadic cases.

• CONCLUSION: Autosomal dominant central areolar choroidal dystrophy, studied in seven families, is caused by an Arg-142-Trp mutation in the peripherin/RDS gene.

Accepted for publication Jan. 9, 1996.

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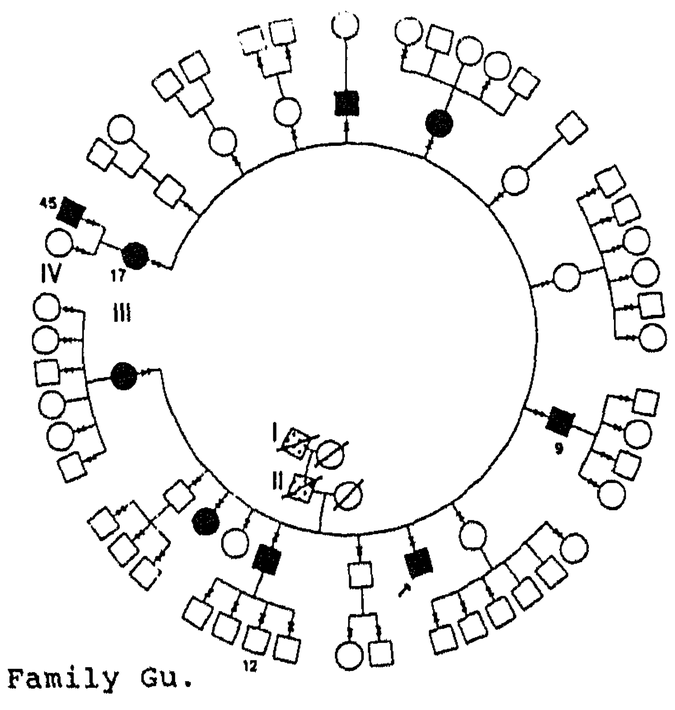
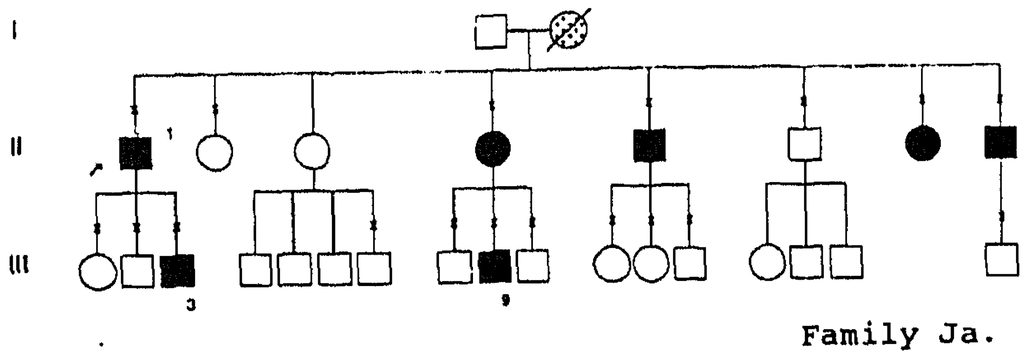
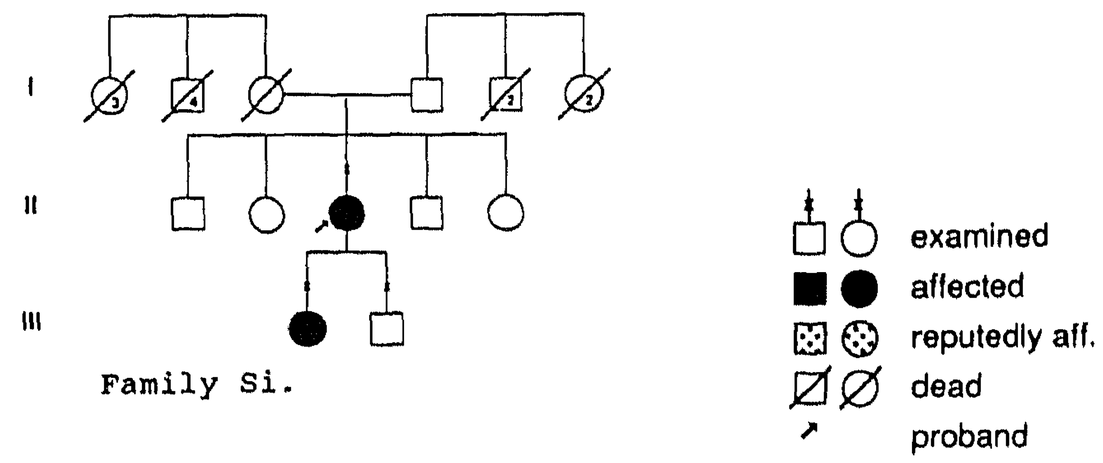
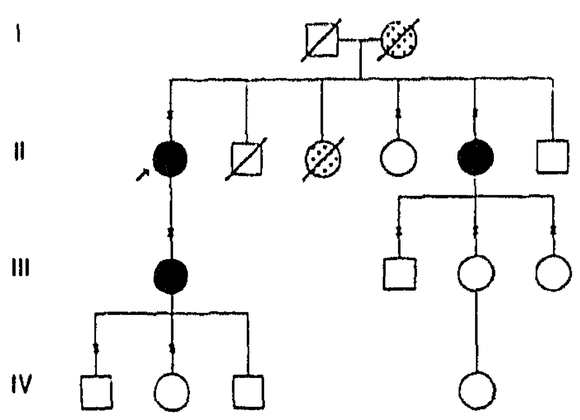
CENTRAL AREOLAR CHOROIDAL DYSTROPHY IS A hereditary disorder of the macula leading to a well-demarcated circumscribed area of atrophy of the pigment epithelium and choriocapillaris. Sorsby[1] was the first to describe the genetic nature of central areolar choroidal dystrophy. In 1955, Sandvig[2] described a family with dominant segregation of central areolar choroidal dystrophy, citing 13 cases spread over four generations. The classic signs and symptoms usually develop between the ages of 40 and 60 years, leading to a central scotoma and visual impairment. Results of retinal functional analysis, by means of electroretinography, electro-oculography, subjective dark adaptation measurement, and color vision examination, are generally normal; however, in advanced cases, color vision is disturbed.[3] The early stages of this disease are characterized by a fine, sometimes hardly detectable, mottling of the parafoveal pigment epithelium. In younger children whose parent has autosomal dominant central areolar choroidal dystrophy, these changes are often not present.[4]

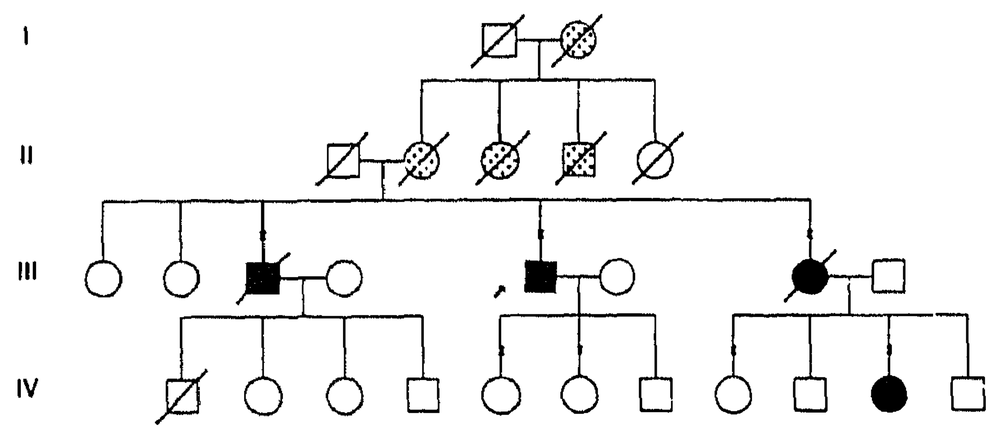
Various mutations in the retinal degeneration slow gene, which codes for peripherin/RDS, have been identified in patients with retinitis pigmentosa, macular dystrophy, pattern dystrophy, fundus flavimaculatus, and butterfly-shaped pigment dystrophy.[5-13] Wroblewski and associates[12] reported mutations in codon 172 in three families with macular dystrophy. In those patients, the area of choroidal atrophy was larger than the area of atrophy found in central

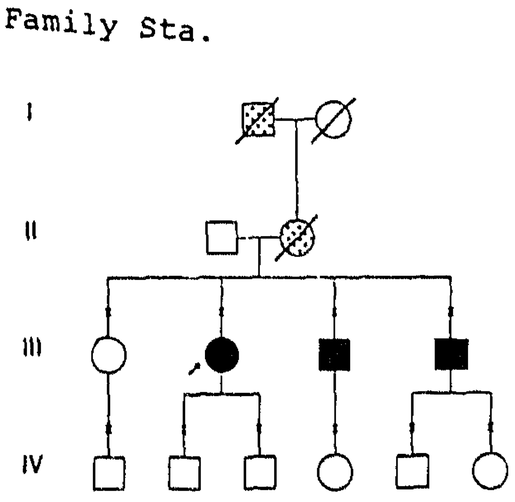












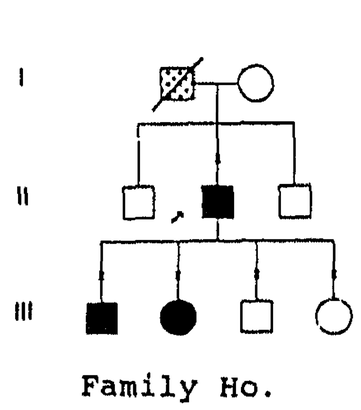


Fig. 1 (Hoyng and associates). Pedigrees of the seven families with autosomal dominant central areolar choroidal dystrophy.

areolar choroidal dystrophy. Furthermore, loss of rod function was found in the dark-adapted rod electroretinogram. The phenotypic resemblance between macular dystrophy and central areolar choroidal dystrophy and the finding that the peripherin/RDS gene is the only gene in which mutations have been shown to be responsible for macular dystrophies prompted us to investigate the involvement of the peripherin/RDS gene in the onset of central areolar choroidal dystrophy.

PATIENTS AND METHODS

IN SEVEN FAMILIES WITH AUTOSOMAL DOMINANTLY INherited central areolar choroidal dystrophy (Fig. 1), we found 30 affected individuals with fundus changes ranging from slight parafoveal depigmentations to the typical end stage, in which there is a well-demarcated area of atrophy of pigment epithelium and choriocapillaris in the posterior pole. From these families, we collected blood samples from ten affected individuals

TABLE

CLINICAL DATA AND RESULTS OF DNA ANALYSIS OF INDIVIDUALS FROM WHOM BLOOD SAMPLES WERE OBTAINED

PATIENT	AGE (YRS)	AGE AT ONSET OF VISUAL COMPLAINTS (YRS)	DISEASE STATUS OF EYES	Arg-142-Trp MUTATION IN THE PERIPHERIN/ RDS GENE*
Autosomal dominantly inherited				
Gu III-2	72		R.E./L.E.: normal	–
Gu III-4	70		R.E./L.E.: normal	–
Gu III-5	69		R.E./L.E.: stage 3	+
Gu III-6	68		R.E./L.E.: normal	–
Gu III-7	36	37	R.E.: stage 3 R.E.: stage 4	+
Gu III-8	65		R.E./L.E.: normal	+
Gu III-10	63		R.E./L.E.: normal	–
Gu III-14	58		R.E./L.E.: normal	–
Gu III-17	53	47	R.E./L.E.: stage 2	+
Ja II-1	65	55	R.E./L.E.: stage 3	+
Si II-3	60	49	R.E./L.E.: stage 3	+
Sta II-2	72	31	R.E./L.E.: stage 4	+
Sta II-4	64		R.E./L.E.: normal	–
Sta II-5	61	50	R.E./L.E.: stage 4	+
Te IV-10	30	25	R.E.: stage 3 L.E.: stage 2	+
Ho II-2	60	54	R.E./L.E.: stage 3	+
Li III-1	55		R.E./L.E.: normal	–
Li III-2	53	52	R.E./L.E.: stage 3	+
Li III-3	50	43	R.E.: stage 3 L.E.: stage 4	+
Sporadic				
A	52	49	R.E./L.E.: stage 3	–
B	66	49	R.E./L.E.: stage 3	–
C	48	47	R.E./L.E.: stage 3	–
D	51	49	R.E./L.E.: stage 3	–

*A plus sign indicates that the mutation was present; a minus sign, that it was absent.

and nine unaffected individuals, who agreed to take part in this study. For inclusion in this study, the status of central areolar choroidal dystrophy had to be stage 2, 3, or 4, as described subsequently. Patients with stage 1 central areolar choroidal dystrophy were not selected because the slight fundus changes in a young person could be coincidental. Because in our previous studies[14] age-related penetrance was estimated to be complete above the age of 50 years (22 of 44 individuals older than 50 years were affected), blood samples from individuals without visual complaints and in whom no fundus changes could be found were collected only if these individuals were older than 60 years. Additionally, we selected four patients whose disease was phenotypically identical to that of the family members but who did not have a family history of central areolar choroidal dystrophy (sporadic cases), in whom the atrophic fundus lesion was found before the age of 50 years. The age at onset of visual complaints and the test results of DNA analysis of the individuals involved in this study are given in the Table. According to earlier studies,[3,14] the development of central areolar choroidal dystrophy was divided into the following four stages: stage 1, discrete

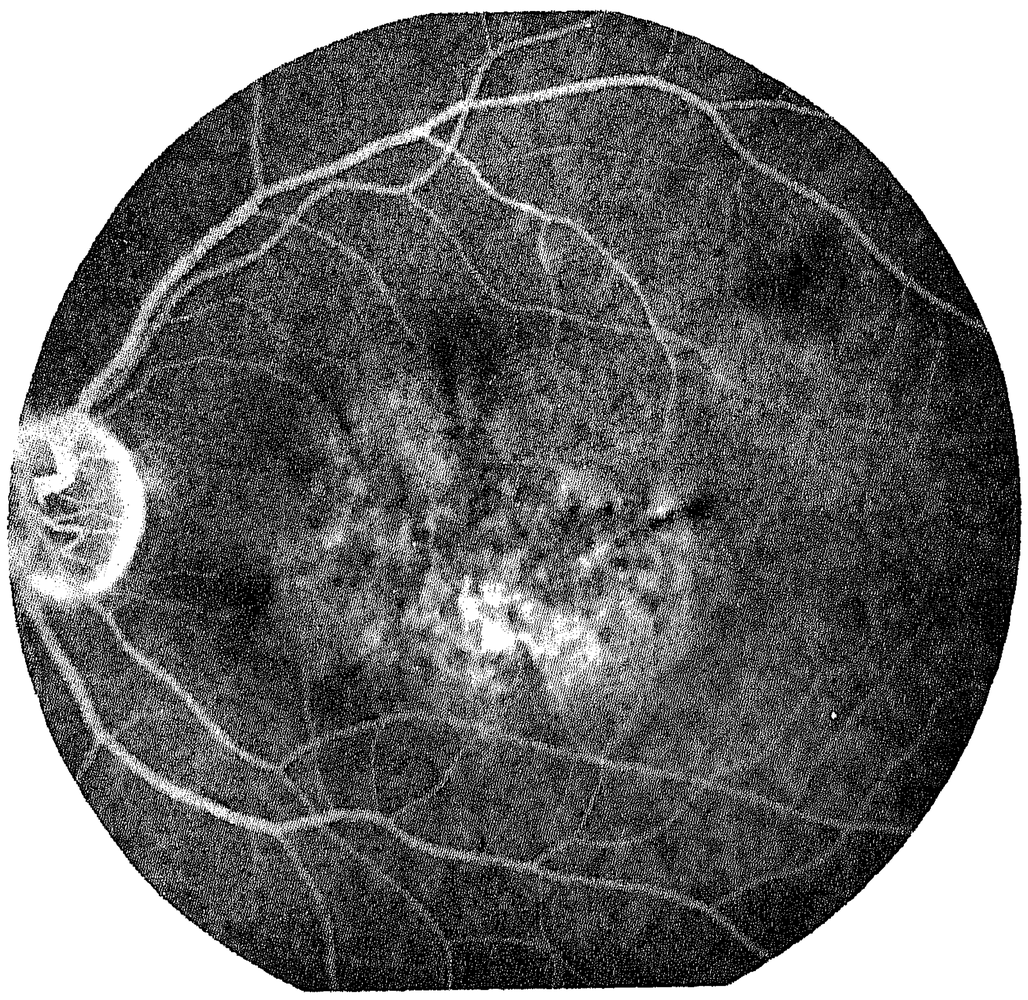


Fig. 2 (Hoyng and associates). Fluorescein angiogram of the left eye of an affected individual (Gu III-17). Hyperfluorescent areas are intermingled with hypofluorescent spots. The choriocapillaris is not yet affected (stage 2 central areolar choroidal dystrophy).

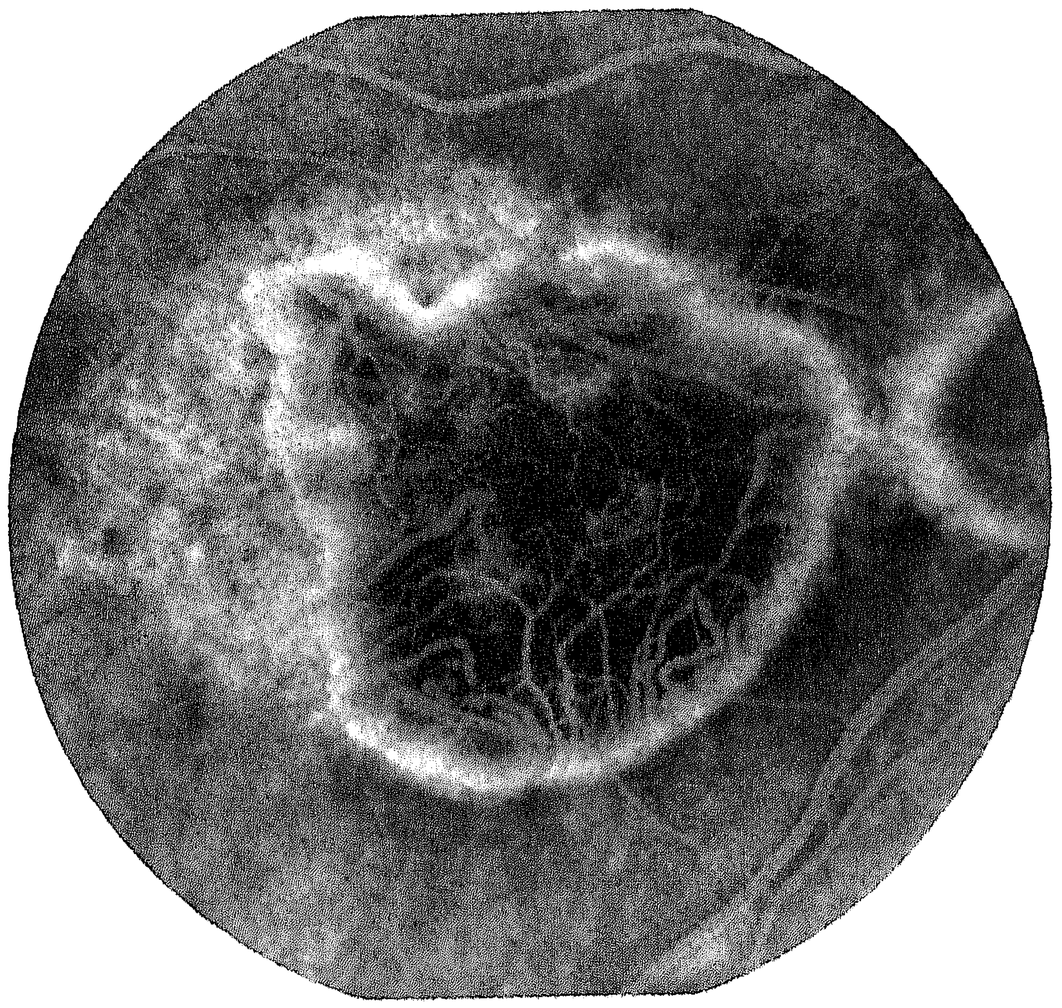


Fig. 3 (Hoyng and associates). Fluorescein angiogram of the right eye of an affected individual. Note the demarcated area of atrophy of the pigment epithelium and choriocapillaris and smaller choroidal vessels. The fovea is affected (stage 4 central areolar choroidal dystrophy).

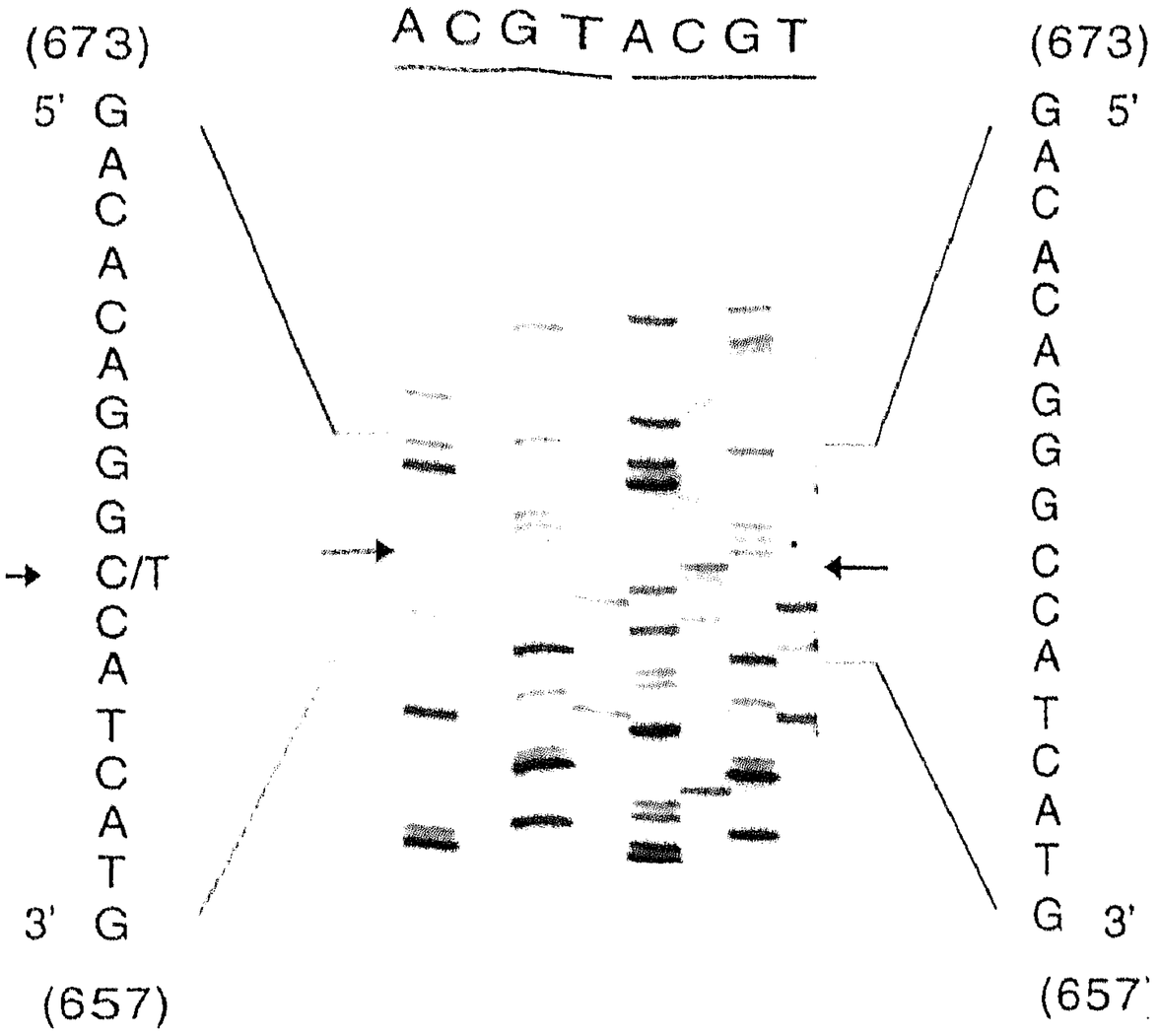
parafoveal retinal pigment epithelial hypopigmentation; stage 2, retinal pigment epithelial hypopigmentation involving the whole parafoveal area; stage 3, stage 2 and parafoveal atrophy of the choriocapillaris; and stage 4, stage 3 and choriocapillaris atrophy also affecting the fovea. The clinical and electrophysiologic data of the family members and of the patients without a family history have been extensively described.[14] Examples of fluorescein angiograms of affected individuals from whom blood samples were taken are shown in Figs. 2 and 3. This study was approved by the institutional review committee of Erasmus University, Rotterdam, The Netherlands.

Genomic DNA was isolated from peripheral blood, as described elsewhere.[15] For mutation analysis, 25 ng of genomic DNA of all family members who were studied and the four patients with sporadic disease was subjected to 30 cycles of polymerase chain reaction. Part of exon 1 of the RDS gene was amplified with the following primers: forward, 5′-GCCAAGTATGCCAGATGGAAG-3′ (derived from the middle of exon 1); reverse, 5′-ATAGCT-CTGACCCCAGACTG-3′ (derived from the 5′ end of intron 1).[16] Polymerase chain reaction conditions were as follows: initial denaturation 5′ at 95 C, denaturation for one minute at 95 C, annealing for one minute at 58 C, extension for one and a half minutes at 72 C, and a final extension for five minutes at 72 C. After amplification, the polymerase chain reaction products were purified on a Microcon-30 column (Amicon, Inc., Beverly, Massachusetts). The same primers were end-labeled with gamma-^{32}P dATP and were used for cycle sequencing according to the protocol provided by the Bethesda Research Laboratories.

To screen for the presence of the mutation in a population sample, an allele-specific oligohybridization test was developed by using the following primers: normal, 5′-GTACTACCCGGGAC-3′, and mutated, 5′-GTACTACTGGGACA-3′. Hybridization and washing conditions were described previously.[17] In total, 200 chromosomes from 100 individuals (50 men and 50 women) were tested.

RESULTS

DNA SEQUENCING OF EXON 1 FOR THE TEN INDEX PAtients of the seven families disclosed a cytosine-to-



Homozygous control: 657- G TAC TAC CGG GAC ACA G -673
Tyr Tyr Arg Asp Thr

Patient: 657- G TAC TAC TGG GAC ACA G -673
Tyr Tyr Trp Asp Thr

Fig. 4 (Hoyng and associates). Top, Nucleotide sequence analysis of exon 1 of the peripherin/RDS gene. On the left, sequence from a patient with central areolar choroidal dystrophy and on the right, sequence from a homozygous control sample. The C-to-T base substitution is indicated by arrows. The numbers 673 and 657 represent positions of the bases in the peripherin/RDS cDNA. Bottom, Nucleotide sequence and the corresponding amino acids of the peripherin/RDS gene between base 657 and 673 in a homozygous control sample and a patient. Nucleotide change and corresponding amino acid substitution are indicated in bold.

thymine change at position 664 of the peripherin/RDS gene.[16] This base pair change results in an amino acid substitution of a tryptophan residue by an arginine residue at codon 142 (Fig. 4). To test whether this base pair change could be correlated with onset of the disease or should be considered to be a polymorphism in the population, we developed an allele-specific oligohybridization test to detect this mutation. In total, 200 chromosomes were tested from the general population. In none of these chromosomes was the mutation detected. Subsequently, blood samples were collected from additional family members and four unrelated patients with a similar phenotype. The mutation was detected in all affected family members who were available (N = 10) and was not present in seven of nine family members without visual complaints.

Two individuals older than 50 years who had the mutation showed no sign of macular degeneration at the time of the initial diagnosis. In one of these patients (Gu III-5; male; age, 69 years), ophthalmoscopy performed in 1988 disclosed no fundus abnormalities. Because of the traveling distance to our institute and because his poor physical condition did not permit him to visit us for reevaluation, we requested another ophthalmoscopic examination. He sent us Polaroid pictures taken with a nonmydriatic camera by his optician (Fig. 5). These pictures clearly showed an atrophic lesion of the posterior pole with sparing of the fovea, confirming our findings on the DNA level. The second individual (Gu III-8; female; age, 65 years) with the mutation agreed to have a reexamination. Visual acuity was 20/20 in both eyes. Ophthalmoscopy and fluorescein angiography showed no abnormalities in the posterior pole.

In the four sporadic cases without family history of central areolar choroidal dystrophy, no mutations were observed in the complete screening of the coding region of the peripherin/RDS gene (data not shown).

DISCUSSION

RETINITIS PIGMENTOSA, AS WELL AS SEVERAL DYSTROphies of the macula, has been associated with mutations of the peripherin/RDS gene. A Pro-216-Ser mutation was demonstrated in a family with retinitis pigmentosa and pattern dystrophy,[8] whereas a Pro-210-Arg mutation was found in a family with both peripheral and macular degeneration.[13] A 3-base pair deletion in codon 153 or 154 has been described in a family with phenotypes of retinitis pigmentosa, fundus flavimaculatus, and pattern dystrophy.[9] Butterfly dystrophy has been associated with Gly-167-Asp, a 2-base pair deletion affecting codons 299 and 300, and a 4-base pair insertion at codon 140.[7,10,11] An Arg-172-Trp mutation was detected in two families,

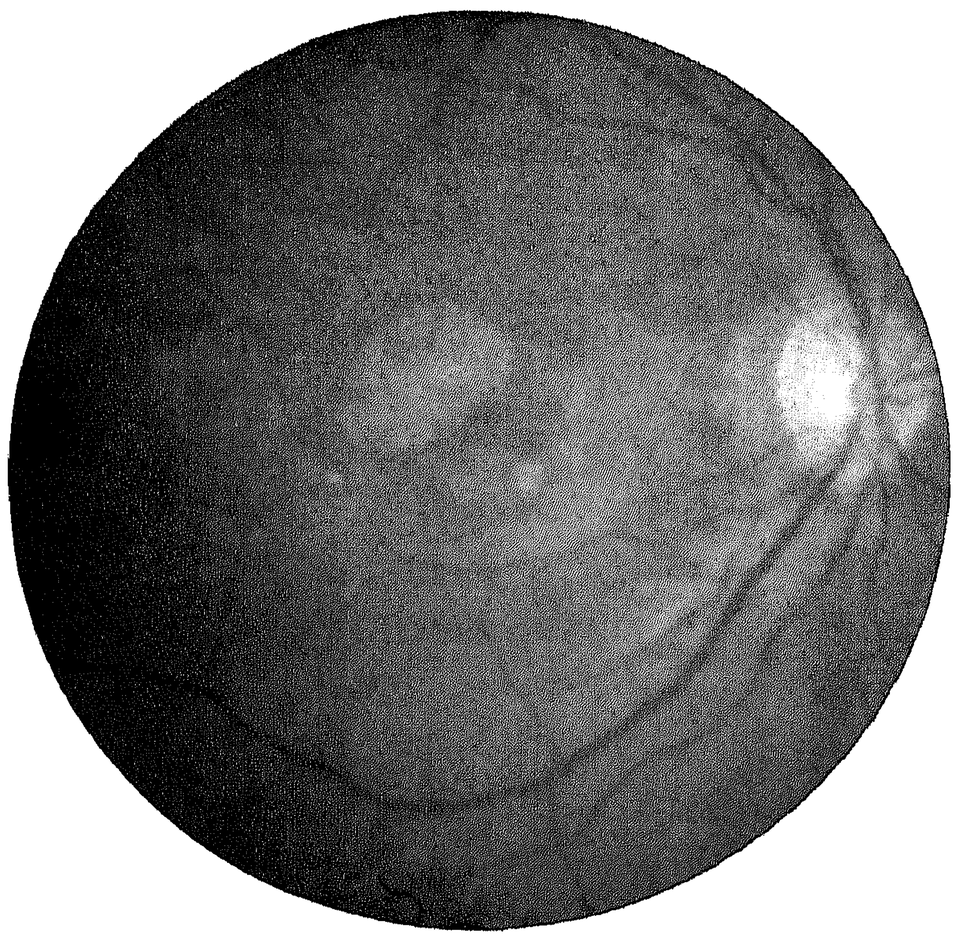


Fig. 5 (Hoyng and associates). After the Arg-142-Trp mutation was found in blood sample analysis, the picture of the right eye of a 69-year-old individual (Gu III-5) established a demarcated area of atrophy of pigment epithelium and choriocapillaris adjacent to the fovea (stage 3 central areolar choroidal dystrophy). Six years before the blood sample analysis, no abnormalities were found in ophthalmoscopic examination.

and an Arg-172-Gln mutation was found in one family with macular dystrophy.[6,12]

The phenotype of central areolar choroidal dystrophy in our families differed clearly from those of fundus flavimaculatus,[9] pattern dystrophy,[8,9] and butterfly dystrophy.[7,10,11] No typical flecks or patternlike hyperpigmentations were observed in our patients. The phenotype of macular dystrophy,[6,12] however, does show resemblance to central areolar choroidal dystrophy. Severe visual loss in siblings from families with macular dystrophy developed before the age of 40 years. Although atrophy of the retinal pigment epithelium was demonstrated early in their lives, in our family members, disabling decrease of vision generally developed in their seventh decade, when the atrophy affected the fovea. Furthermore, the atrophic macular lesions in our patients were smaller and not connected to the optic disk.

In all the affected family members from whom blood samples were obtained, a change from C to T was found at position 664, resulting in a change of codon 142 from CGG to TGG in the peripherin/RDS gene. This base pair change leads to a substitution from arginine to tryptophan (Arg-142-Trp). Similar to most other mutations that have been reported so far for other macular dystrophies, the mutation in central areolar choroidal dystrophy is located in the second interdiskal loop of the peripherin molecule.[18] Three other mutations are located in the transmembrane segments 1, 3, and 4.[18] The function of the peripherin molecule is unknown, but it has been suggested that it functions as an adhesion molecule, stabilizing outer segment disks through homophilic or heterophilic interactions across the interdiskal space. The second intradiskal loop of the peripherin protein is located extracellularly and probably is involved in these interactions.

In one 65-year-old patient (Gu III-8), the Arg-142-Trp mutation was found, although no macular changes were observed on fluorescein angiography. However, as in her brother (Gu III-5), in whom the atrophy became manifest between the ages of 63 and 69 years, central areolar choroidal dystrophy could still develop at an older age.

In the four sporadic cases, no mutation was found in the regulatory region of the peripherin/RDS gene. Either the phenotype was caused by a mutation in the noncoding region of the peripherin/RDS gene or by a mutation in a different gene. In these families we found no other affected individuals. Therefore, it was not possible to determine whether the disease in these patients is linked to the peripherin/RDS gene.

In conclusion, we found an Arg-142-Trp mutation in seven families with autosomal dominant central areolar choroidal dystrophy. Although from our earlier studies penetrance was estimated to be 100% above the age of 50 years, we found one individual who carried the mutation but had not developed any signs of central areolar choroidal dystrophy at the age of 65 years. Her affected brother developed signs of central areolar choroidal dystrophy between the ages of 63 and 69 years.

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